THE DOWN REGULATION OF MGLUR5 ATTENUATES THE REACTIVE PHENOTYPE IN ALS SPINAL CORD ADULT ASTROCYTES EXPRESSING THE SOD1G93A MUTATION

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by a selective death of upper and lower motor neurons (MNs). ALS is a very complex and multifactorial disease which etiopathogenesis is not completely understood. Moreover, several in-vitro and in-vivo studies demonstrated that damage within MNs is sustained by the degeneration of non-neuronal neighboring cells such as microglia and astrocytes (1). Thus, to unveil the specific role of the single CNS cells involved in ALS, turn out to be crucial in order to better understand the etiopathogenesis and to design a more targeted therapy to counteract the pathology.

In this frame, Group I metabotropic glutamate receptors (mGluR1, mGluR5) seems to play a role, being found to be largely over-expressed and functionally altered in different experimental model of ALS (2, 3, 4). Indeed, we recently demonstrated that knocking-down mGluR1 or mGluR5 significantly prolongs survival and ameliorates the clinical progression in the SOD1G93A mouse model of ALS (5, 6).

Based on our results, the aim of this work is to investigate, the effects of a genetic mGluR5 down-regulation on the reactive phenotype of astrocytes in ALS. To achieve this goal we used spinal cord adult primary astrocyte cell cultures from SOD1G93A mice, SOD1G93A_mGluR5+/- mice, heterozygous for the mGlu5 receptor, and age matched control mice.

To perform this study we planned to exploit a multidisciplinary approach spanning from intracellular calcium concentration [Ca2+]i assay, immunofluorescece (IF) and cytofluorimetric analysis, ELISA assays, to investigate the astrocyte neuroinflammatory secretome and astrocytes-MNs co-cultures.

The spectrofluorophotometer experiments, performed with the FURA-2 fluorescent dye, showed a significantly higher [Ca2+]i, in SOD1G93A astrocytes compared to the respective controls, in both basal and stimulated conditions with the Group I mGluRs selective agonist 3,5-DHPG (150 vs. 280 nM and 200 vs. 350 nM respectively; $p<0.001$). As expected, the mGluR5 down regulation significantly reduced the [Ca2+]i evoked by 30 µM 3,5-DHPG in astrocytes from SOD1G93A_mGluR5+/- mice compared to SOD1G93A astrocytes (275 nM; $p<0.01$). Interestingly, also the basal [Ca2+]i was significantly diminished in astrocytes with half expression of mGluR5, respect to SOD1G93A astrocytes (225 nM; $p<0.01$). These data indicate that the constitutively elevated [Ca2+]i in ALS astrocytes can be, at least in part, modulated by halving mGluR5 expression. In parallel, IF analysis pointed out interesting issues; in particular, we detected a significant decreased expression ($p<0.05$) of several markers linked to astrogliosis, such as GFAP, Vimentin and S100β, when comparing adult astrocytes form SOD1G93A_mGluR5+/- mice with SOD1G93A mice. Moreover, we found a marked decrease ($p<0.01$) in the expression of LC3, as hallmark of authophagy activation, in SOD1G93A astrocytes heterozygous for mGluR5 respect to

SOD1G93A astroglial cells. Unexpectedly, the mGluR5 genetic down regulation also translates in a significant lower cellular localization of misfolded-SOD1 protein when comparing SOD1G93A_mGluR5+/- versus SOD1G93A astrocytes ($p<0.05$), supporting a possible link between the mGluR5 and the SOD1 protein misfolding/degradation.

The results obtained so far demonstrate that a lower constitutive level of mGluR5 has a significant positive effect on astrocytes bearing the SOD1G93A mutation, supporting the idea that mGluR5 may be considered as a potential pharmacological target for a cell specific therapeutic approach in ALS, aimed at preserving MNs death by modulating the reactive phenotype of the neighboring astroglial cell.

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